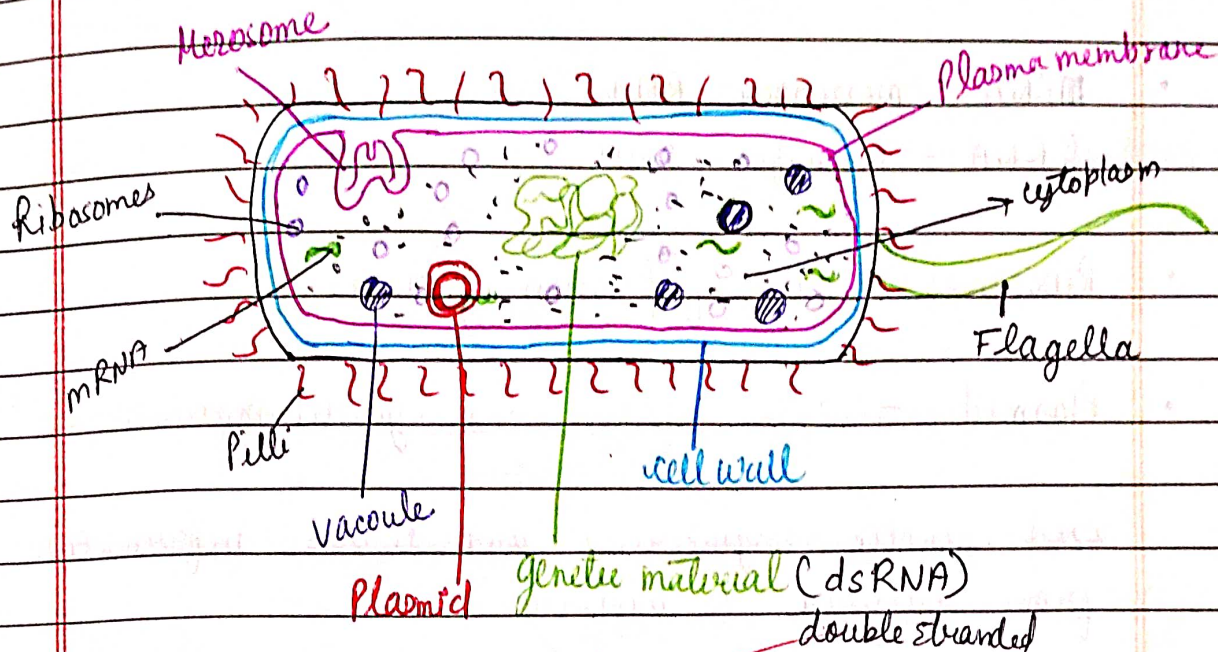
Waheguru ji

11/2/2022

Page No.
Date / 201

# Structure of Bacterial cell.

Merosome
Plasma membrane
Ribosomes
cytoplasm
mRNA
Flagella
Pilli
cell wall
vacoule
Plasmid
genetic material (dsRNA)
double stranded

- Plasma membrane
  semi permeable
  Transport of waste material

- Infolding
  merosome
  bacterial respiration is takes place by mesosome.

- Flagella - locomotion movement of bacterial cycle.

- Pilli
  Thin short hair like Auile SSDNA.
  help in attachment to any structure

Page No.
Date / 201

1/4/22

- Cytoplasm
  dense material and provide ridgidity to baiterial cell

- Genetic materia (ds DNA).
  In center

- MRNA - messenger RNA.
  t RNA - Transfer RNA.

- Ribosomes - site of protien synthesis

- Plasmid - extra chromosomal genetic material

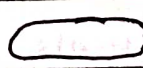
  used genetic engineering and shares information from bacteria to bacteria.

# MICROBIOLOGY

study of microorganism under the microscope

History of Microbiology -

Father of modern Microbiology - Louis Pasteur
Fermentation
sterlisation
Pasturisation

Robert Koch - Germ theory of disease.

Antonie Van Leeuwenhock - Compound microscope

Zoseth Lister - Antiseptic surgery.

Edward Jenner - vaccine for pox virus.

classification of Microorganism -

On the basis of shape and size.

1. cocci — eg. streptococcus (o Monococcus, oo Diplococcus, oooo Tetracoccus) — spherical
2. Bacilli — eg. salmonella — Rod
3. Comma (Vibrio) — eg. vibrio cholerae — comma shaped
4. Spirilla — eg. spirillum minus — spiro

⇒ On the basis of nutrition

Autotrophic bacteria - Bacteria that prepare food by its own.

Hetrotrophic bacteria - Bacteria that depends upon other.

1. Autotrophic bacteria

a) Phototropic Bacteria - use sunlight for preparing food.

e.g. - Cyanobacteria, Green bacteria, purple bacteria

b) Chemotropic bacteria - Bacteria use chemical energy for prepare food.

•) chemotrops (Inorganic compounds use)
eg. Sulphur bacteria
Nitrifying bacteria
(Nitrosomonas.

•) chemoorganotrops - Bacteria use organic compound

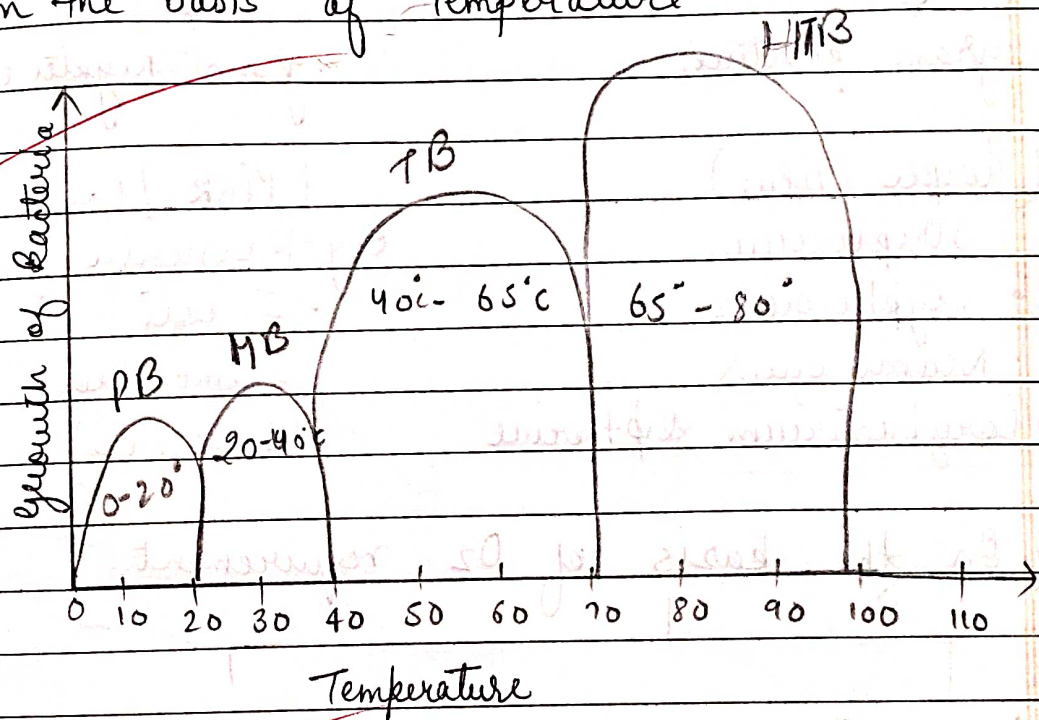
e.g. Purple non - sulphur bacteria.

2. Heterotrophic Bacteria -

- **Saprophytic Bacteria** (Grow and dead and decaying organic matter) Fungus, Bacillus Cerous
- **Symbiotic Bacteria** (do nitrogen fixation, mutual association with plants) Eg. Rhibozokium.
- **Parasitic bacteria** (disease causing bacteria) Eg. Salmonella.

⇒ On the basis of Temperature -

Growth of Bacteria (y-axis) vs Temperature (x-axis: 0 10 20 30 40 50 60 70 80 90 100 110)

PB 0-20°
MB 20-40°
TB 40°c - 65°c
HTB 65° - 80°

0-20 - Psychrotrophic bacteria
20-40 - Mesophillic bacteria
45-60/65 - Thermophillic bacteria
65/70 - 80/90 - Hyperthermophillic bacteria.

8/4/22

⇒ On the basis of PH (0-14)

Acidic
0 - 7 | 0·65 - Acidophillic bacteria eg. Lactobacillus

7-neutral | 6·5 - 7·5 - Neutrophillic eg. E-coli

7-14 Basic. | 8 - 14 - Basophillic / Basilophillic bacteria eg. Vibrio chlerae

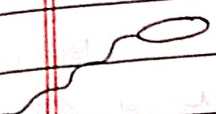
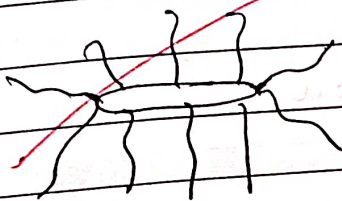
⇒ On the basis of staining

gram positive — (Purple / Blue)
eg. • streptococcus
• styphococcus
• Neumococcus
(Rod) Corybacterium diptheriae

gram negative — (Pink / Red)
e.g • Nersseria (cocci)
• E-coli
• Salmonella
• (Neisser's)

⇒ On the basis of $O_2$ requirement

Aerobic bacteria
Requires oxygen
two types -
obligate aerobes - eg. Vibrio cholerae which grow only in presence of $O_2$
Microaerophillic bacteria - are those grow best in the presence of low $O_2$ tension eg. Helicobacter

Anaerobic bacteria
grow in absence of $O_2$
Facultative anaerobes - $O_2$ presence absence) Microaerotolerant bacteria (If we give them 5-10% $O_2$ they can also grow).

4. On the basis of flagella

a) - Atrichous - no flagella eg Beggiatoa

b) - Monotrichous - eg. cholera vibrios

c) - Lophotrichous eg. Pseudomonas.

d) - Amphitrichous eg. spirillum

e) - Peritrichous eg. E-coli, Salmonella

CHAPTER : 3.

Page No.
Date / 201

# STERLIZATION

The term sterlization is defined as the process by means of which an article surface medium is made free from all living microorganisms including spores

sterlisation is the complete inactivation of all forms of microbial life.

Sterlising agent
physical, chemical.

sterlisation Method.

Physical method | mechanical | chemical method.

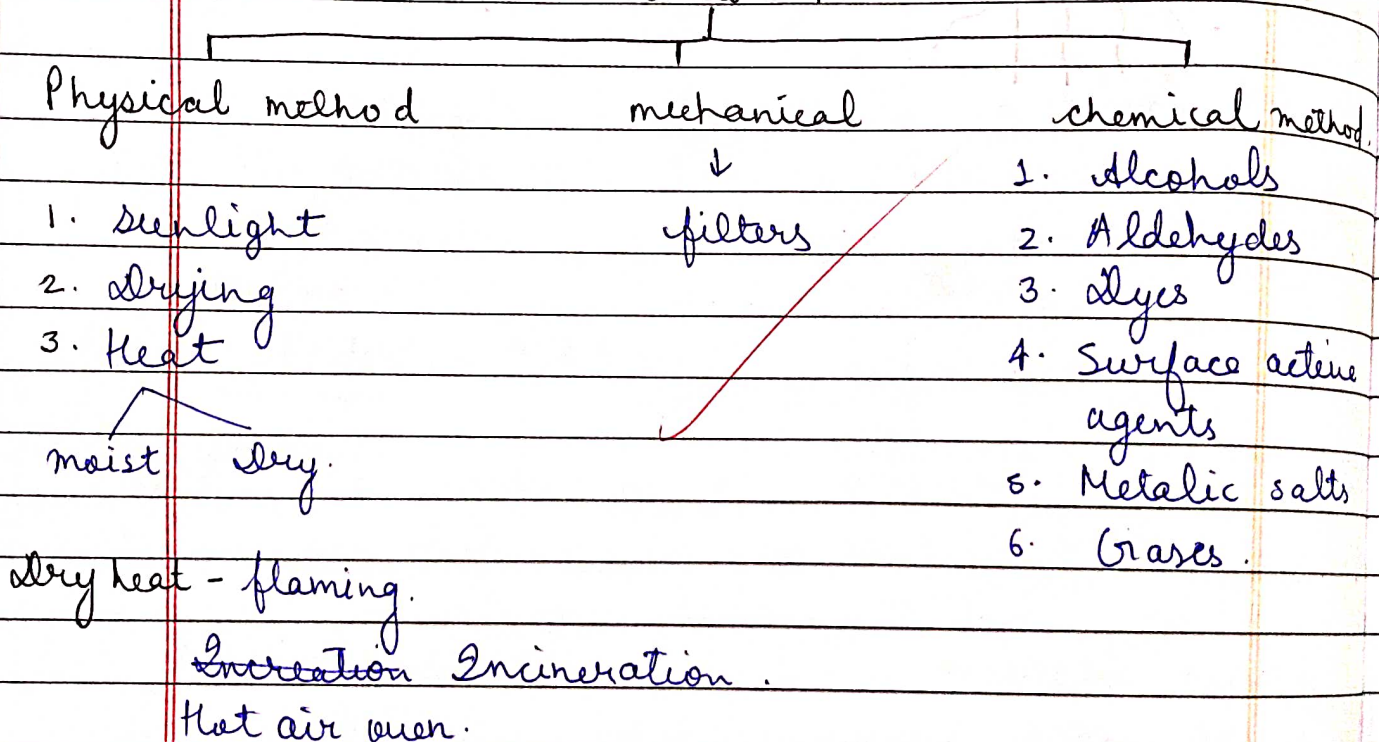
Physical method:

1. sunlight
2. drying
3. Heat
   - moist
   - dry.

mechanical:
↓
filters

chemical method:

1. Alcohols
2. Aldehydes
3. dyes
4. Surface active agents
5. Metalic salts
6. Gases.

dry heat - flaming.
~~Increation~~ Incineration.
Hot air oven.

Moist heat - 1) Pasteurization
2) Boiling
3) Autoclave

chemical method and their agents.

1) Alcohols – ethyl alcohol

2) Aldehydes – Formaldehydes

3) dyes – Acridine dyes, Aniline dyes.

4) surface active agents - Soap, detergents

5) Metalic salts – Mercuric chloride.

6) Gases – Ethylene oxide, formaldehyde.

Mechanical method
↓
Filters
membrane filter
chamber land filter.

## Disinfection

The term disinfection is defined as the process of distruction of vegetative forms of pathogenic organisms which are capable of producing infection but not necessary

Disinfection · are usually reserved for inanimate objects.

Page No
Date / 201

Antiseptic's

Antiseptics are the chemical substance can be safe chemical disinfectant to skin / mucous membrane and are used to prevent infection by inhibiting the growth of bacteria

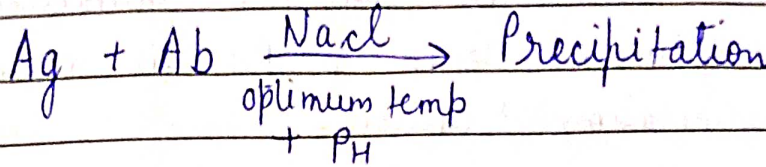
"Wahiguru ji"

Page No.
Date / 201

Chapter - 13

# Antigen - Antibody Reactions
# Serological Reactions

## Precipatation Reaction

$$Ag + Ab \xrightarrow[\text{optimum temp} + P_H]{NaCl} \text{Precipitation}$$

when a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen - antibody complex forms an insoluble precipitate.

Ag - Ab Reaction's / Serological Reactions -

Serological is the study of serum it is the science that studies antigen antibody Reactions of the body using a serum specimen.

Many diagonistic laboratory test are based on antigen antibody reactions. These test to detect antibodies aganist the infecting microorganism provide a useful means of indirect diagnosis.

These test are of special value for those organisms which cannot be isolated and cultured in the laboratory.

Serological reactions are simple rapid and specific

The test results are reliable and reproducable.

Types of serological Reactions -

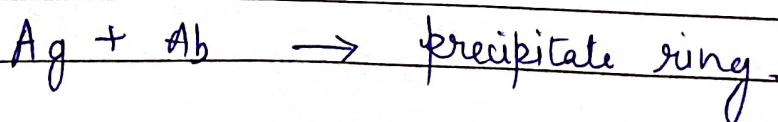
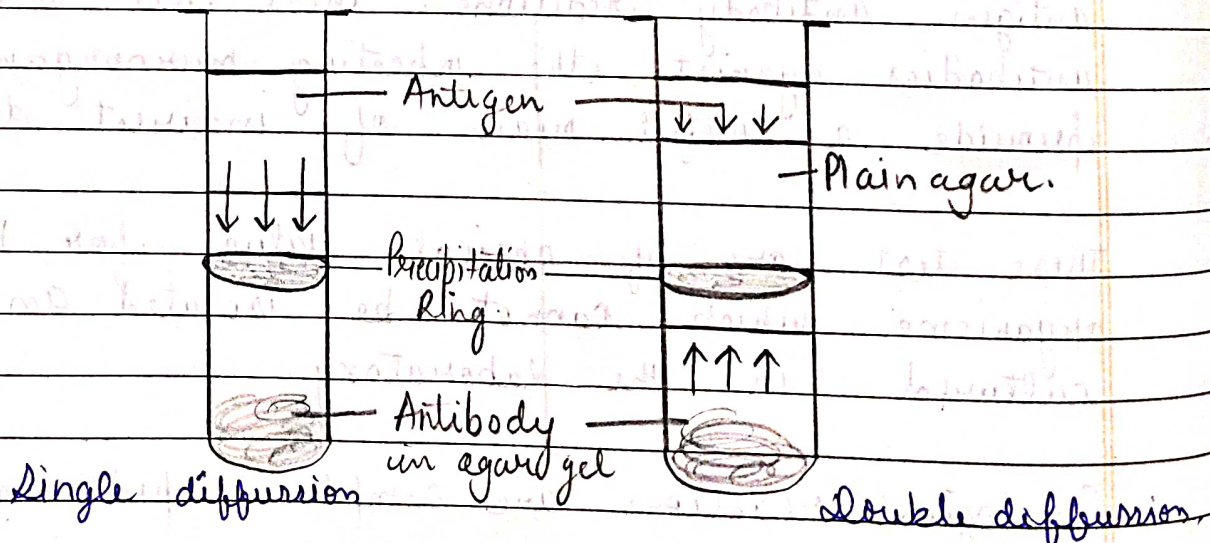
1. Precipitation Reaction - when antigen and antibody reaction with each other in the presence of NaCl and optimum temp & PH it results into precipitation reaction.

b. Types of slide percipitation Reaction :

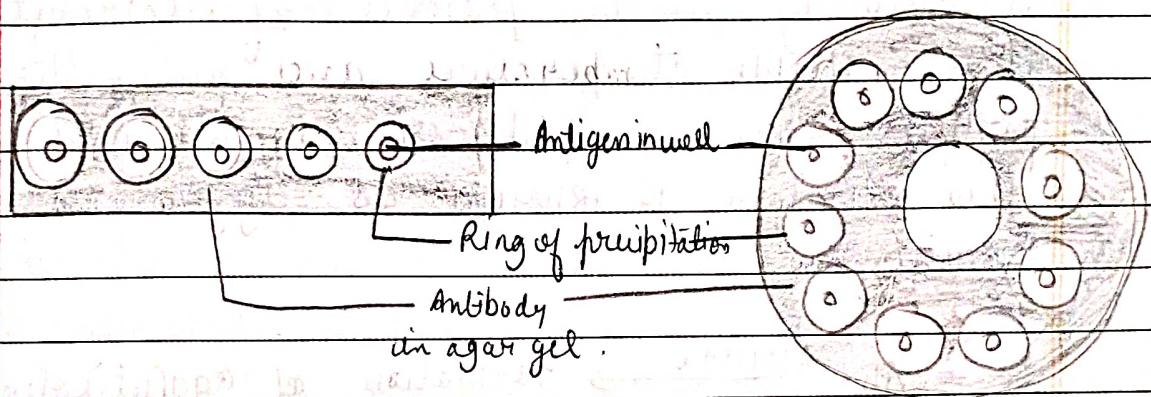
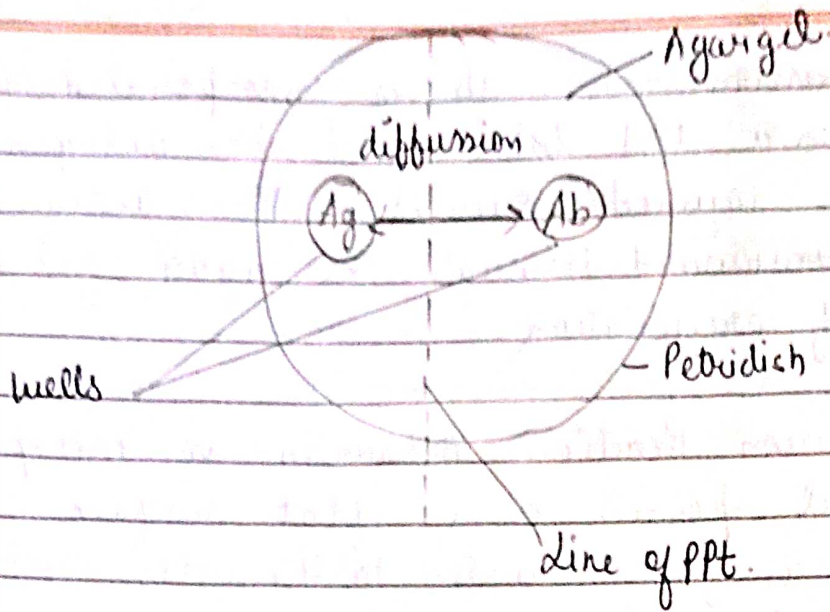
i) Test Tube precipitation Reaction - The antibody is incorporated in gel, above which is placed in a column of plain agar. The antigen is layred on top of this. [Double Diffusion]

Ag & Ab move towards each other through intervening column of plain agar and form a band of perecipitate at point where they meet at optimum proportion.

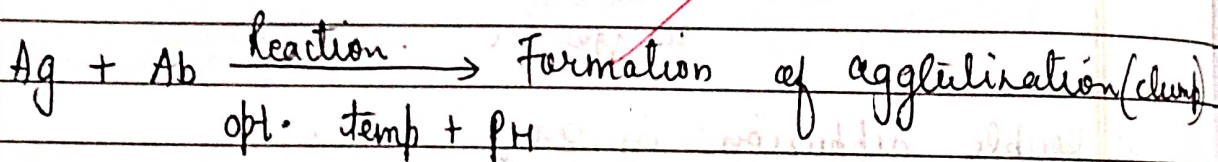
Antigen
Plain agar.
Precipitation Ring.
Antibody in agar gel
Single diffusion
Double diffussion.

Ag + Ab → precipitate ring.

b) Single diffusion - The ab is incorporated into agar gel in a test tube and the antigen solution is layered over it. The antigen diffuses downward through the agar gel forming a line of precipitation.

Immuno diffusion Reaction. Antiserum is incorporated
a) in agar gel poured on a flat surface like slide. The antigen is added to the wells on the surface of the gel. It diffuses radially from the well and forms ring-shaped bands of precipitation, concentrically around the well.

Antigen in well
Ring of precipitation
Antibody in agar gel.

b) Double diffusion in 2-D -
Agar gel is poured on a slide and wells are cut using a template. The antiserum is placed in the central well, & different antigens in the surrounding wells.
If two adjacent antigens are indentical, the lines of precipitate formed by them will fuse and if they are ~~identi~~ non-indentical the LOP will cross each other and indicated by spur formation.

2) AGGLUTINATION REACTION:

When a particulate antigen mixed with the its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated. This reaction is known as agglutination reaction.

Ag + Ab $\xrightarrow[\text{opt. temp + pH}]{\text{Reaction}}$ Formation of agglutination (clump)

eg. Blood grouping slide test.

Agglutination occurs only when antigen and ~~ab~~ antibody are mixed ~~with each other~~ at optimal proportions.

## NEUTRILISATION REACTION

when an antitoxin combines with the a toxin the biological effects of the toxin are neutralised rending it harmless to animals since toxin is an antigen in solution. It is also precipitated.

Toxin , Anti-Toxin Neutrilization can be measured in vivo(out) and invitro (in).

virus neturilisation Test - Neutralising coat viruses once coated the viruses cannot penetrate cells in the body. Test serum is mixed with a suspension of infectious virus particles of the same type as that of the indinisual in suspected to have been infected.
The virus suspensions are then enclaulate into ampunated at or tissue culture that supports the growth of virus.

1. Neutrilisation test in IV - (Invitro) [Schick Test
Agar gel in the precipitation.
It is employed to detect the production of toxins by Corynebacterium diphtheriae.

2. Anti-Streptolysin O (ASO) test - It demonstrates that the antitoxin present in serum of patient infected with streptococcus contains Anti-streptolysin O, that neutralises the streptolysin O.

immunosorbent - an absorbing material specific for one of the components of the reaction (Ag-Ab) for eg cellulose / agarose, solid phase / polystyrene.

Date / 201

ELISA - Enzyme Linked Immunosorbent Assay
Based on Ag - Ab reaction.

Principle - It is plate based immuno acid. It work on the principle of antigen - antibody binding. It is based on enzymatic colour reaction. It detects and indentifies substances such as peptides protiens harmone and antibodies.

Sample for ELISA -

1. Serum
2. CSF
3. Sputum
4. Urine
5. Stool

Types of ELISA

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA

1. Direct ELISA - (direct ELISA).

Conjug is was out as not free

Page No.
Date / 201

## Indirect ELISA

Microtiter well coated with Antigen

↓

And serum (contain primary antibody that is enzyme linked).

↓

wash - To remove unbound antibodies

↓

Add substrate (chromogen) for the enzyme

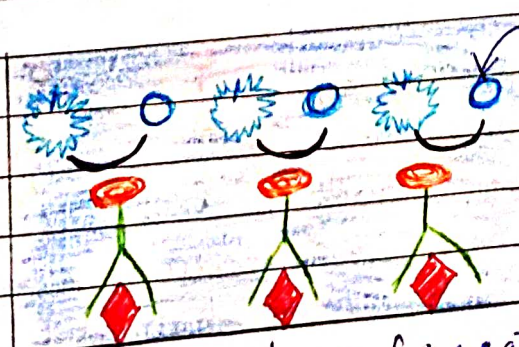
↓

~~Enzyme cleaves substrate to produce color.~~

Virus sample on the surface.

Antigen with enzyme conjugate attached to viral antigen

~~Conjugate is washed out as ag is not free to bind the conjugate~~

Substrate and enzyme interaction create color change for detection.

substrate.

Enzyme is not there to act on substrate

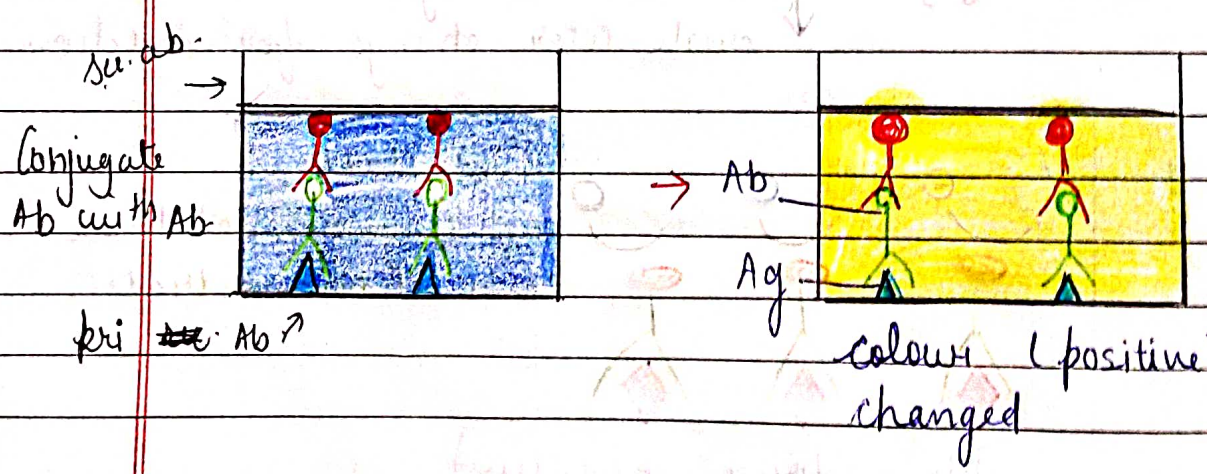
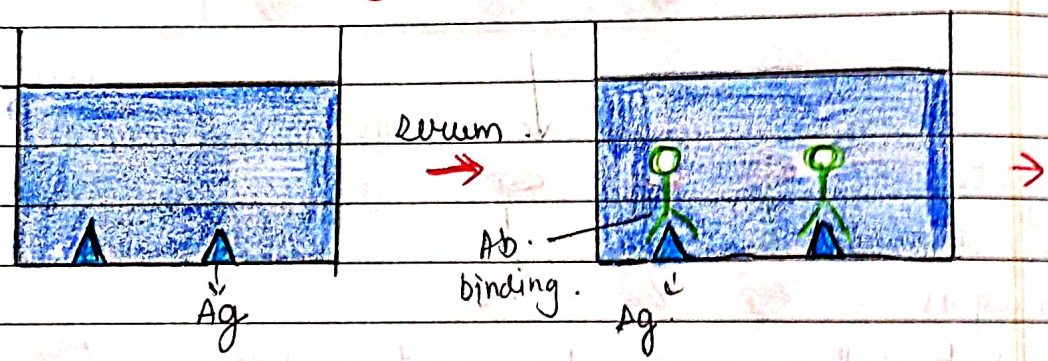
No colour (negative)

It is ... Produces ... catalise ... phos... as ...

a/e +ve'

# Indirect ELISA

Microtiter well coated with Antigen
↓
Add serum (contains primary Ab)
↓
Wash - To remove unbound antibodies.
↓
Add secondary Ab (Enzyme linked)
↓
Wash - To remove unbound secondary Ab.
↓
Add substrate (chromogen) for the enzyme.
↓
Enzyme cleaves substrate to produce color.

Page No.
Date / 201

# Sandwich ELISA.

Microtiter well coated with capture Antibody

↓

Add serum (contains Ag to be detected)

↓

wash to remove unbound antibodies.

↓

Add enzyme linked antibody

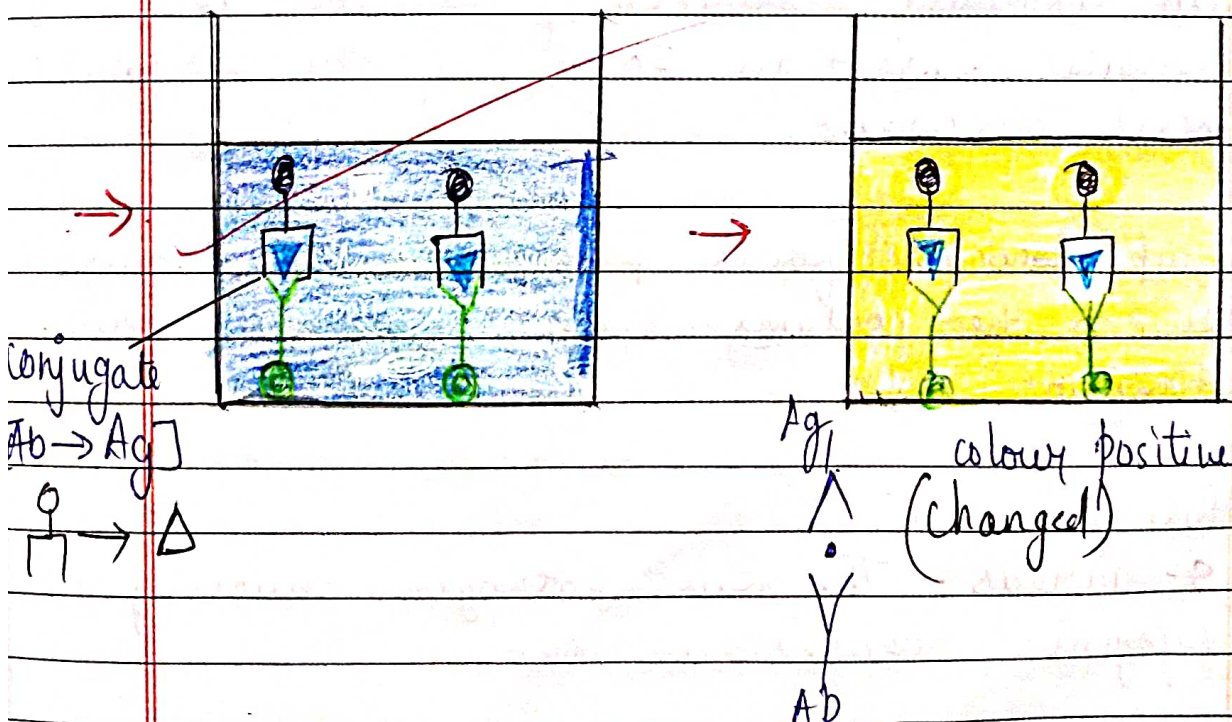
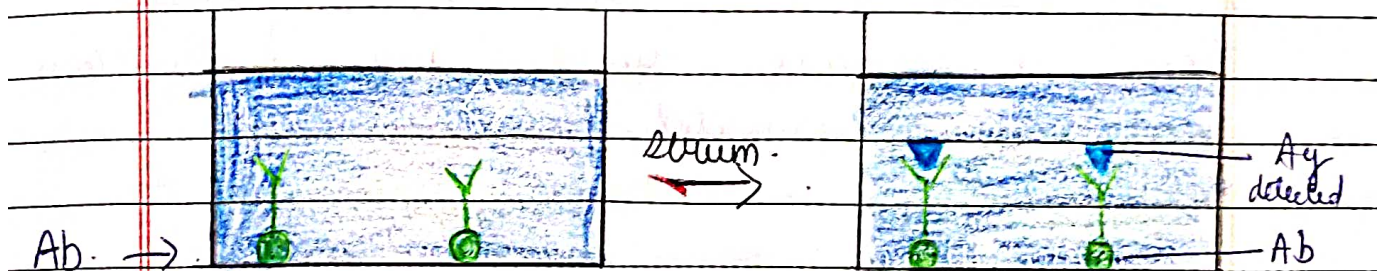
↓

Add substrate (chromogen) for the enzyme.

↓

Enzyme cleaves substrate to produce color

Ab. →
serum. →
Ag detected
Ab

→
conjugate
Ab → Ag]
→ Δ
→
Ag
Ab
colour positive (changed)

20/05/22

CH: 20

# STAPHYLOCOCCUS

It is a gram positive cocci occuring in groups like cluster, because there is a cell division takes place in all planes. It is a hemolytic cocci.

It is a commonest positive organism of pyogenic lesions in men.
They are catalyse positive aerobes or facultative anerobes.
Grow in presence of 7.5% 10% NaCl

These are spherical, ovoid, gram positive cocci. Their size approximately measure 1μm in diameter.

## Source of infection -

Human patient and carrier due to are to commonest source of infection in hospital more than 50% of the hospital staff is carriers.

styphilococcus food poisining mostly occurs due to the contamination of food from food handlers

## Pathogenesis -

1. S- aureus - The main pathogen causeing pyogenic causes in men

2. S. epidermidis - It may lead to septismia and subaccute endocarditis.
It may produce minor lesion like stich, abscess.

3. S. Saprophyticus - mainly seen in genito urinary mucus membrane and skin
It act an opportunistic pathogen

## Cultural characterstics -

S- aureus - grows easily and optimum easily temperature of 37°c and PH of 7.4 in the presence of O2.

In nutrient agar media on 24 H incubatio produces golden yellow circular convex smooth 2-4 nm shiny and opaque colonies.

In Macconkey media - colonies are small and pink.

## Lab diagnosis -

Sample - blood.
Gram staining - appear blue / purple cocci in cluster

culture -

Page No.
Date / 201

22/05/22

# STREPTOCOCCUS / STREPTOCOCCI

streptococci are gram positive arranged in chains or pairs.

**characterestics** -

1. These are gram positive.
2. Non - sporing
3. Non - motile
4. Capsulated (tend to more pathogenic)
5. Aerobic & normally facultative anaerobic (c can be grow both in absence / presence of oxygen)
6. The individual cocci are spherical or ovoid approximately measure 0.5-1 µm in diametere and characteristically arrange in chains varying in length.

**Source of Infection** -

Human patient & carriers are the commonest source of infection.
streptococcus infection are less frequent in childrens less than 3 years old but more frequent in school age children

Nasal carriers are the most dangerous source of the organism.

## Pathogenesis -

Acute infection of respiratory tract -

i) Streptococcal sore throat - This is the most
* common disease, may be localised as acute tonsillitis or may involve the pharynx more diffusely.

ii) skin infection - Empetigo & pyoderma manifest as localised infections with induration tenderness & pus point.

iii) Acute Tonsillitis

iv) Sepsis

Septicemia.

Abscess in internal organ.

Bacteremia - infection in blood.

B. Toxin mediated infection

i) Scarlet fever

ii) Toxic shock syndrome.

C. Non Suppurative infectious sequelae.

- Rheumatic fever

Hypersensitivity reactions.

## Biochemical Reactions -

Most sugar like glucose, lactose, sucrose maltose manitol are fermented & production of acid takes place but no gas.

They are catalase negative.

# Streptococcal Diseases.

| Group A | Lesions |
| --- | --- |
| Streptococcal Pyogenes | |
| Suppurative infections | |
| a) Respiratory Tract | Sore throat, tonsillitis, sca[rlet] fever |
| b) skin and soft tissue manipulation | Impetigo, pyoderma, erysip[elas], infection of wound, burns & chronic skin infections like eczema psoariasis. |
| c) Genital infections | Puerperal sepsis. |
| d) Deep infections | Bone & joint infections, Lymphadenitis, septicemia, acute endocarditis. |
| Non-Suppurative Lesions. | |
| a) Heart | Rheumatic Fever. |
| b) Kidney | Acute post streptococcal glomerulonephritis. |
| Streptococcal faecalis | Endocarditis |
| streptococcal faecium | urinary tract infection |
| streptococci viridans | Endocarditis. |
| group B streptococci | Neonatal infections (pneumonia, mengitis respiratory disease. |

S
S -
S -
S -
S.
S. S
S.
S.
S.

Page No.
Date / 201

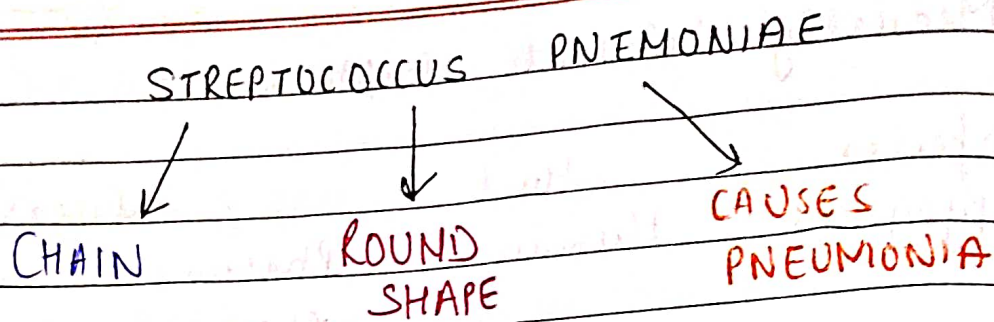
Medically relevant streptococci :-

| Species | Host | Disease |
|---|---|---|
| S- Pyogenes | Human | Pharyngitis cellulitis erysipelas. |
| S- agalactiae | Human cattle | Neonatal mengigitis & sepsis. |
| S- dysgalactiae | Human animals | endocarditis. bacteremia. pneumonia, mengitis. respiratory infections. |
| S. gallolyticus | human animals | biliary or urinary tract infections. endocarditis. |
| S. anginosus | human animals | subcutaneous / organ abscesses meningitis. respiratory infections. |
| S. sanguins | human | endocarditis, dental caries. |
| S. suis | swine | meningitis. |
| S. mitis | human | endocarditis |
| S. mutans | human | dental caries. |

scarlet fever.
erysipelas

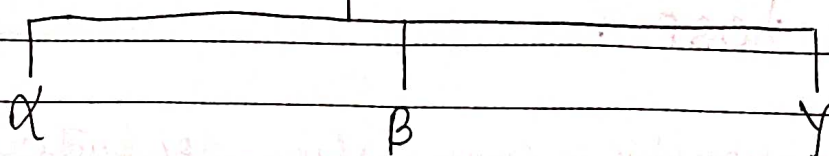
Page No.
Date / 201

STREPTOCOCCUS PNEMONIAE

- CHAIN
- ROUND SHAPE
- CAUSES PNEUMONIA

- Round Bacteria
  grow in chains
  Most common cause of - community acquired pneumonia

Pneumonia symptoms:

→ Fever
→ Labored breathing
→ ) cough
) fever
) chills
) loss of appetite
) Nausea & vomiting especially in small children
) ~~shake~~ rise in temp
) chest pain when coughing
) severe weaking.
) Bluish skin
) Body aches.
) Meningitis associated with pneumonia

Page No.
Date / 201
STREPTOCOCUS
gram positive cocci in chains, catalase negative
α
β
γ
Partial hemolysis
Complete hemolysis
No hemolysis
optochin sensitivity
complete hemolysis
grow 6.5% NaCl
No
Yes
viridans strep (unencapsulated)
S. pneumoniae (encapsulated)
No
Yes
S. Bovis
Enterococci
S. Faecium
S. Faecalis.
No
Yes
group B
S. agalactiae
group A strep
S. pyogenes

Page No.
Date / 201

Laboratory Diagnosis.
Sample - ) Blood sample.
) Sputum.
) urine.
) CSF.

steps -
1. Direct microscopic examination - of bacterial smear. - After smearing the sample is forwarded by gram staining and as a result Blue colour coccus appears in chain like structure

2. culture - Nutrient agar media.
After 24 hours incubation the colonies are large (2-4 mm diametere), round smooth shiny and often pigmented. styphilococal pigment is believed to be a carotenoid and its derivated located in cell membrane

Most strain form the golden yellow pigment or orange yellow pigment colonies.

2. Blood Agar Media -
colonies are almost similar to those on nutrient agar. They produce beta haemolysis which is best scene with rabbit or sheep blood.

3.) Mac Conkey's Agar - colonies are very small and pink due to lactose fermentation

4 ~~Biom~~ Biochemical Reactions / Test

They ferment sugar and produce acid but no gas.

They are catalyse positive

Reduce nitrates to nitrites ($NO_3 \rightarrow NO_2$)

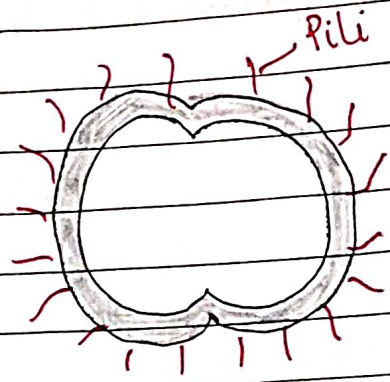
Prevention for Infection.

?

25/05/22

Date / 201

# Neisseria
(gram -ve cocci)

Neisseria Meningitis | Nisseria gonorrhea (Gonnorrhea)



## Neisseria Meningitis -

These are gram negative diplococci commonly colonize the nasopharynx of healthy people. It was first isolated by weichselbaum in 1887 from spinal fluid of a case of meningitis. It cause acute purulent meningitis & also called as cerebrospinal meningitis or cerebral fever.

1st proven

## characterstics -

These are gram negative oval cocci. 0·6 - 0·8 μm occur typically in pairs. with adjacent sides flat or concave bean shaped and the encess of the pair parallel and not in line as in the pneumococcus.

These are non-motile and capsulated.

Source of infection -

- Human patients are the commonest carriers. Nasal carriers are the most dangerous source of the mechanism.

Pathogenesis -

Meningococcemia presents with acute fever chill, cutaneous rash, Hemmorages often associated with metastatic involvement of joint, ears, lungs & myocardium.

Diagnosis -

a) specimen and sample
→ CSF or Blood.

1st process: detection of organism by smear examination and immunological examination.

2nd process

b) Biochemical Test - Catalase and oxidase positive ferment glucose and maltose producing acid but no gas.

c) Culture - Grows well media enriched with blood serum or specially in presence of 10% $CO_2$
colonies in solid media are moist elevated round, convex and translucent

colonised in solid media are moist, smooth elevated brown convex and translucent

Examination - Direct

1. gram staining
2. growth in chocolate agar medium and higher Martin selective media.
3. observing fermentation of glucose
4. oxidative test positive

Indirect examination

1. Compliment fixation Test
2. Flucent Ab. Technique.
3. ELISA
4. Muckocytosis.

'Waheguru ji'

27/05/22

TB Causes - Myobacterium tuberculosis

Known as - Acid Fast bacteria (appears red after staining)

## GRAM POSITIVE BACILLI

Diag - Acid fast staining and Ziehl Neelson staining.

## TUBERCULE BACILLI

Myo-bacterium tuberculosis is also known as Tubercule Bacilli. It is a pathogenic bacteria species in the genus mycobacterium and causitive agent of Tuberculosis.

First discovered on 24th march 1882 by Robert Koch who recieved the noble prize in medicine for this discovery in 1905. The bacterium is also known as 'Koch Bacillus'.

characterstics -

1. It is a non-motile, non-sporing, non-capsulated bacillus measures 2-3 X 0·2 - 0·4 μm. arranged singly or in groups.
2. This is an obligate arrow (only grow in the presence of oxygen) hence acid fast staining is used for their staining

Source of infection -

Mycobacterium Tuberculosis cause human lesion like pulmonary Tuberculosis.

Infection in human is transmitted mainly by inhalation of bacilli in moist droplets of respiratory secretions from the open pulmonary tuberculosis.

occassionly infection occurs by ingestion of infected milk (cow) Mycobacterium - Bovis found in milk of cow.

The bacilli are more resistant to drying and chemical disinfectants. Moist heat at 100°C kills them readily. It's unusual cell wall rich in lipids (Mycolic acids) is a likely responsible for this resistant

cultural characteristics - These are aerobes grow slowly colonies usually appear in 2-3 weeks and may some times require 8 weeks incubation with optimum temperature 37°C at PH 6.4-7.0. They grow only in special enriched media (additional nutrient added) containing eggs, potatoes, serum and meat extract Lowenstein Jensen (L.J Medium) is used in for their cultivation

Pathogenicity -
Primary infection - Primary infection may occur in any organ like lungs, tonsils, skin or intestine. In children the usual site is in the lungs. In most cases the primary infection is asymptomatic of undergoes spontaneous cleaning by resolution

Page No.
Date / 201

## Distinguishing b/w M. Tuberculosis & M. Bovis

| Test | M Tuberculosis. |
|---|---|
| 1. Morphology | Slender, long and usually covered. |
| 2. Staining | Barred or beaded |
| 3. Growth | Eugonic |
| 4. Action of glycerol in media | Growth enhanced. |
| 5. Colony | dry rough raised, wrinkled colour - creamy white / buff colour |
| 6. Biochemical Reaction | |
| i) Niacin production | positive |
| ii) Nitrate reduction | positive |
| iii) TcH selective action | negative. |
| 7. Animal Pathogenecity | |
| i) Progressive disease in Rabbit | negative or mild lesion |
| In guineapig. | positive progressive & ultimately fatal disease |

## Drug concentrations in antimicrobial susceptibility testing for myobacteria.

| Drug | Lowenstein-Jensen | Middle brook 7H10 |
|---|---|---|
| Isoniazid. | 0.2, 1 | 0.2, 1 |
| Streptomycin | 2 | 4 |
| Rifampin | 1 | 40 |
| Ethambutol | 2 | 2 |
| Ethionamide | 5 | 20 |
| Kanamycin | 5 | 20 |
| Capreomycin | 10 | 20 |
| Cycloserine | 20 | 30 |
| Pyrazinamide | 50 | |

Solid. M
Egg
1) Low
2) LJ w
3) Wa
4) Vibr

Indentification

staining – Mycobacteria are acid fast (means they stain red / Pink) because of the presence of N- glycol -muramic (mycolic) acid in their cell walls.

Zeil' Neelson stain is most commonly employed acid fast technique

bacterial cell wall of myobacterial cell.

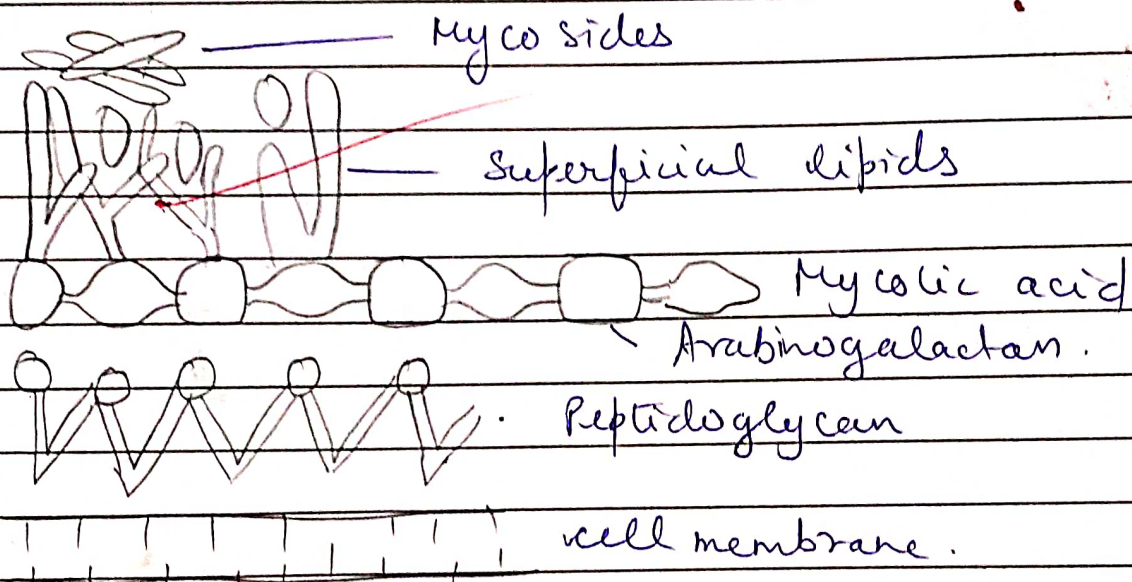
Mycosides

Superficial lipids

Mycolic acid

Arabinogalactan.

Peptidoglycan

cell membrane.

Media for Mycobacterial culture specimen.

Solid Medium — Components.

Egg based

1) Lowenstien Jensen (LJ) – eggs, defined salts glycerol

2) LJ with pyruvic acid – LJ + pyruvic acid.

3) Wallenstien – Egg yolk, 2.5% glycerine

4) Petragnani – Cogulated eggs, egg yolk whole milk.

Agar-based -

1) Middle brook 7H10 - Salts, oleic acid, albumin, catalase, glycerol

2) Middle brook 7H11 - MB 7H10 + Casein hydrolysate

Liquid -

1. Dubo's medium - Bovine serum albumin, asparagine, casein, hydrolysate, certain salts with Tween.

2. ~~Middle brook 7H9~~ -

30/05/22